Cholinergics (PNS)

Introduction

This penultimate lesson ties many previous lessons together, and so assumes you have mastered several lessons prior to this one. This lesson assumes you have studied general physiology, and are competent with the synapse and the neuromuscular junction. This lesson also assumes you know the general effects of the cholinergic system on the heart, eye, and lungs from lesson #8: *Intro to Autonomics*. Finally, this lesson assumes you have mastered the intracellular second messenger systems presented in lesson #7: *Receptors and Second Messengers*. Now we assemble everything you have already learned to start talking about autonomic drugs. We'll talk about acetylcholine metabolism, the neural synapse, and the specific types of receptors (subtypes) and how medications can affect them.

The Cholinergic Synapse: Acetylcholine

The effect of acetylcholine depends on the receptors it activates, the subtype of receptor, and the organ being innervated. This section is NOT about the receptors, but about the synaptic cleft, and the neurotransmitter cycle for acetylcholine. We are looking at the generalities of any chemical signaling between a neuron releasing acetylcholine and the receiver of that chemical signal. There are nicotinic receptors that are ionotropic and depolarize the postsynaptic cell. There are also metabotropic receptors that initiate second-messenger cascades. For this immediate discussion, that acetylcholine "does its thing" is all the detail you need about postsynaptic cell activity. Stay focused on the acetylcholine neurotransmitter cycle, metabolism, synthesis, and packaging. Follow along with Figure 9.1.

We begin by describing the cycle. We begin the cycle where degraded neurotransmitter in the form of **choline** is brought into the presynaptic cell, a process called **uptake**. Once inside the presynaptic cell, **reformation** of the acetylcholine is carried out—choline and acetyl-CoA are used to synthesize new acetylcholine. Acetylcholine molecules are then packaged into vesicles and shipped to the axon terminus, where they wait. **Depolarization** of the presynaptic cell causes the opening of voltage-gated calcium channels, the influx of calcium, and the fusion of the vesicles with the plasma membrane of the cell. This results in **exocytosis** of the vesicles and **release of acetylcholine** into the synaptic cleft. Acetylcholine then "does its thing" on the postsynaptic cell. **Acetylcholinesterase**, the enzyme that degrades active acetylcholine into inactive choline, acts as the termination signal. The degradation of acetylcholine to choline means that no acetylcholine receptors can be activated without another depolarization and release of new acetylcholine.

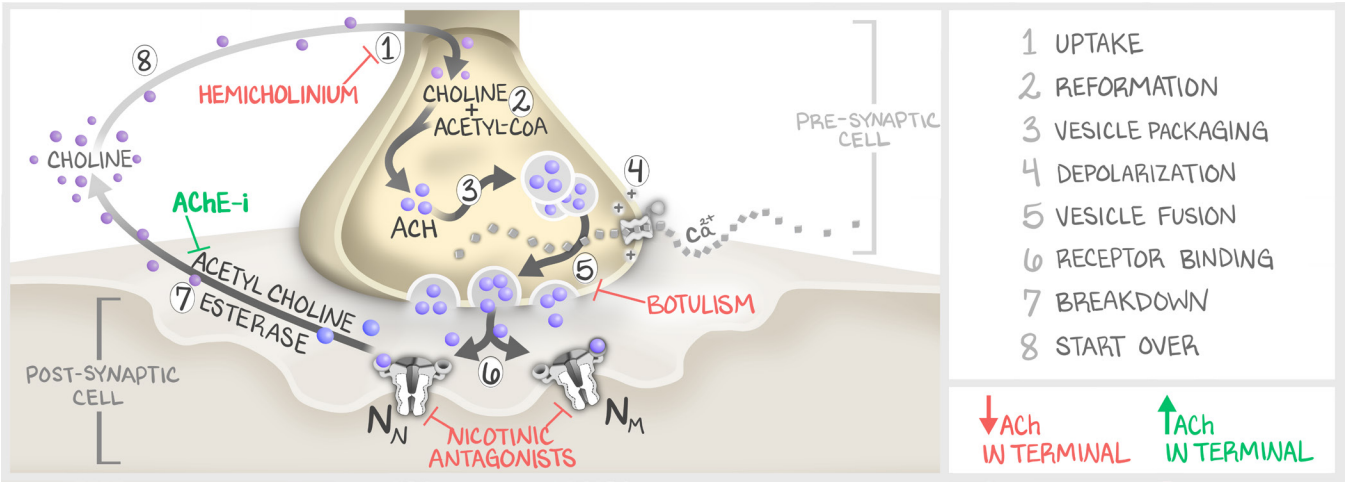

Figure 9.1: The Synaptic Cleft and Acetylcholine Metabolism
The eight-step process mirrors the pattern of the neuromuscular junction studied in General Physiology. Prepackaged vesicles full of acetylcholine await depolarization of the axon terminus. Depolarization leads to calcium influx, vesicular fusion, and release of acetylcholine into the synaptic cleft. Acetylcholine can act on ionotropic receptors (skeletal muscle, preganglionic synapse) or metabotropic receptors (effector synapse). Acetylcholinesterase degrades active neurotransmitter to choline, and choline is reuptaken into the presynaptic terminal to start the process over again. Botulism toxin prevents vesicular fusion and release; hemicholinium prevents reuptake of the acetylcholine. Acetylcholine receptors can be direct acetylcholine receptor agonists or indirect agonists (acetylcholinesterase inhibitors).

Manipulation of acetylcholine levels in the synaptic cleft alters the degree of response of the postsynaptic cell—more acetylcholine means more response for longer duration; less acetylcholine means less response and shorter duration.

One way to **increase acetylcholine activity** (i.e., increase acetylcholine concentrations) is to **prevent metabolism** (degradation) of acetylcholine. Only one enzyme is responsible for that, and **antagonizing acetylcholinesterase** (acetylcholinesterase inhibitors) will achieve more acetylcholine remaining longer in the synaptic cleft if the presynaptic cell releases acetylcholine. With an increase in terminal ACh, the system will be stimulated. The addition of the drug behaves just like an agonist to the ACh receptors themselves, but **only in innervated tissue**. In reality, the drug does not have any active site on the receptor at all, so has no effect if a neuron does not release ACh. Therefore, AChE inhibitors are pro-ACh-receptor function, but don't act on the receptor, so are called **indirect agonists**.

There are several viable options for **decreasing acetylcholine activity** (i.e., decrease acetylcholine concentrations), and they all have to do with the uptake, synthesis, packaging, and release of acetylcholine. Any drug that did this would reduce the ACh activity of the system, but would do so without influencing the receptor itself. The effect of the drug would appear as an AChR antagonist, even though it never has any active site on the receptor itself. Therefore, drugs that do this would be considered **indirect antagonists. Botulism** acts at the nerve terminal, preventing vesicle fusion, preventing release of ACh. **Hemicholinium** prevents reuptake of choline and the synthesis of ACh in presynaptic neurons.

We also have drugs that target the receptors, and do not need an intact neuron to release acetylcholine to exert an effect. **Direct-acting agonists** bind to and activate cholinergic receptors. Direct-acting agonists can be specific for nicotinic or for muscarinic, and are often specific to a subtype. **Direct-acting antagonists** bind to the receptor and prevent its activation.

But there are two types of acetylcholine receptors—nicotinic and muscarinic. Nicotinic receptors are ionotropic and are present in skeletal muscle cells at the neuromuscular junction and at the ganglionic synapse between first- and second-order neurons. Muscarinic use intracellular second messengers. There is a special class of medications called **ganglionic blockers** which block **nicotinic acetylcholine receptors** of the ganglia. We discuss those last.

Nicotinic Receptors in Detail

There are two types of nicotinic receptors: N_M and N_N. The $\mathbf{N_M}$ type is in **skeletal muscle**, and was discussed in General Physiology under excitable cells, and will not be discussed further in this lesson. The $\mathbf{N_N}$ type is in the membrane of postganglionic neurons. Activation of the postsynaptic nicotinic AChR by presynaptic release of ACh causes **depolarization** of the postsynaptic cell.

**Technically, the SNS also uses N_N at the preganglionic synapse and the synapse with the adrenal medulla, but we're keeping this discussion focused on the PNS only.*

The $\mathbf{N_N}$ **receptor** has **no intracellular messenger**. Instead, it is **ionotropic**; activation results in equal conductance to $\mathbf{Na^+}$ and $\mathbf{K^+}$, driving the membrane potential around that receptor to be between the equilibrium potentials for both Na^+ and K^+. That is a depolarizing stimulus. When many are activated at the same time, the postsynaptic cell reaches threshold, and it depolarizes. When this second-order postganglionic nerve depolarizes, its axon carries that depolarization to the next synaptic junction with the effector organ, where release of ACh activates **muscarinic acetylcholine receptors.**

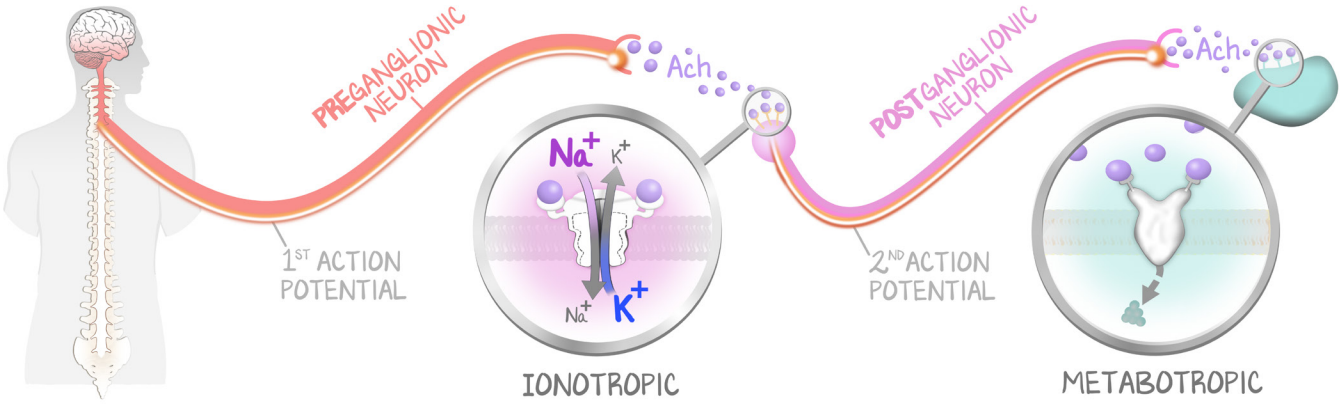

Figure 9.2: Acetylcholine Receptors
First-order neuron depolarizes, and carries the action potential down its axon to the preganglionic synapse. The depolarization causes release of ACh into the ganglion synapse, activating ionotropic acetylcholine receptors and depolarizing the postganglionic neuron, which carries its action potential to the effector organ synapse, releasing ACh into the synapse, stimulating the effector organ cells via metabotropic muscarinic acetylcholine receptors.

Muscarinic Receptors

Muscarinic receptors come in three major forms: M_1, M_2, and M_3. (Strictly speaking, there are five, but I really only want you to learn M_2 and M_3, so M_4 and M_5 will never be mentioned. M_1—the first to be discovered—is mentioned only for the sake of inclusion.) They are how the effector organs respond to a parasympathetic stimulus. They are also the target of our therapeutics.

	WHAT IT DOES	HOW	G PROTEIN	SECOND MESSENGER
M_1	Exocrine glands, CNS	Glands secrete	G_q	IP_3, DAG, Ca
M_2	**Nodal heart**	**Opposes cAMP-PKA**	**G_i**	**↓ cAMP, ↓ PKA**
M_3	Lacrimation, defecation, urination, salivation, pupillary constriction	Smooth muscle contraction	G_q	IP_3, DAG, Ca
M_3	Blood vessels	Endothelial cells stimulated to release nitric oxide	??	??

Table 9.1: Muscarinic Receptor Subtypes

M_1-AChRs affect primarily glandular secretion, which is exocrine function. They work through an intracellular messenger system that involves a G protein-coupled transmembrane protein—the G_q-PLC-IP_3-DAG-Ca system. I mainly ignore M_1 except to know that it's there.

M_2-AChRs are the **receptors of the heart**. These receptors are on **nodal cells only**, and don't influence contractility. The nodal cells are stimulated by G_s and inhibited by G_i. **M_2**-AChRs are **G_i-coupled**, meaning that they inhibit the cAMP-PKA-phosphorylation pathway. Activation of M_2-AChRs inhibits the cAMP pathway and causes the **heart rate to slow** by reducing myocyte automaticity and slowing conduction through the AV node (see Cardiac: Electricity #5: *Anti-arrhythmics* for more details).

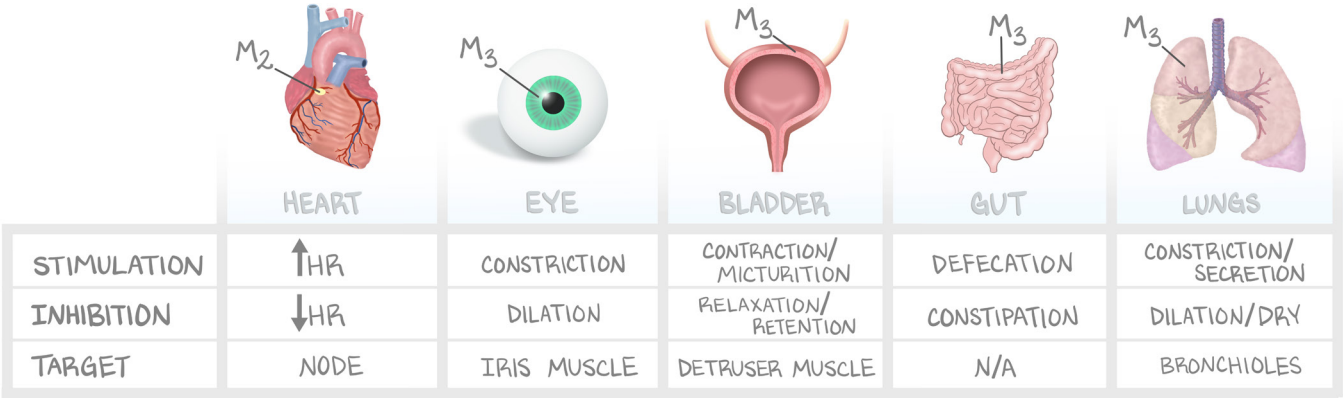

Figure 9.3: Muscarinic Subtypes and Their Effects
M_2 receptors are on the heart; stimulation slowing heart rate and inhibition increasing heart rate. M_3 receptors are on multiple organs. On the eye, M_3 receptor activation leads to constriction, inhibition dilation. On the bladder, M_3 receptor activation leads to contraction of the detrusor muscle and urination, inhibition causes urinary retention. On the gut, M_3 receptor activation leads to defecation, inhibition causes constipation. And on the lungs, M_3 activation leads to bronchoconstriction and secretion, inhibition leads to bronchodilation and drying of secretions.

M_3-AChRs have **two** mechanisms. The first is like M_1 in that it causes **smooth-muscle contraction** through the **G_q-PLC-IP_3-DAG-Ca** system. Calcium is released, and the smooth muscle contracts. Where those smooth muscles are will determine the overall effect—constriction will occur. Smooth-muscle contraction of the detrusor muscle causes urination; smooth-muscle contraction of the iris dilator causes pupillary constriction; contraction of rectal muscles causes defecation. Also, as a memorization point, M_3 also relaxes sphincters, increasing the elimination. M_3-AChRs seem also to be **excretory**, causing the classic syndrome of **SLUDGE** when overstimulated—salivation, lacrimation, urination, defecation, GI upset, emesis.

The second mechanism is unexpected. While it's true that the parasympathetic nervous system has no direct innervation of blood vessels, it's a bit false to say there's no effect on blood pressure from parasympathetic tone. The PNS innervates the endothelial cells of arteries. M_3 activation of endothelial cells results in nitric oxide release, leading to vasodilation—we leave the details of this mechanism out of autonomics because the nitric oxide pathway is more relevant in Cardiac: Coronary Artery Disease #4: *Chronic Ischemic Heart Disease Pharmacology*.

Cholinergic Drugs #1: Direct-Acting Muscarinic Agonists

Direct-acting agonists are used to get things done when the innate ability to do them has been lost. They are **direct-acting** and so the **drug itself** binds to and activates the receptor. Technically, this means that these drugs **are effective** even if there is no neuron releasing acetylcholine. Being **direct**, these drugs are **reversible** and **competitive**. A common side effect is **vasodilation** through activation of the endothelial "nitric oxide mechanism" if they end up systemic. The highest-risk is bethanechol. With vasodilation comes orthostatic hypotension and reflex tachycardia.

To reduce side effects, we try to manufacture drugs that are either subtype-specific or that are delivered to the organ we want to stimulate, reducing the systemic distribution to organs where we don't want it. For example, in a patient with glaucoma the goal is **pupillary constriction**. We should want to use a medication that affects only the eye, avoiding systemic side effects. **Pilocarpine eye drops** are used for such a purpose in patients with narrow-angle glaucoma. Pilocarpine is never used intravenously because the impact on the eye would be minimized and systemic activation of M_3 would lead to a wet mouth and wet pants.

Sometimes the only way to garner a therapeutic effect is to distribute the drug systemically and tolerate side effects. **Bethanechol** ("*Beth Anne, call your bladder and tell it to move*") is used for **neurogenic bladder**. To induce micturition, the detrusor muscle must contract. In neurogenic bladder there is a compromise of the innervation to the detrusor muscle. So to induce detrusor muscle contraction, bethanechol stimulates M_3 receptors, acting through the G_q-PLC-DAG-IP_3-Ca system (intended effect). The colon is also stimulated by M_3 activation, and in almost equal efficacy to the bladder. Which means that if the bladder is slow and bethanechol gets it to move, but the gut was doing fine, the added "go" signal to the bowel can cause diarrhea.

Don't be tricked—memorize this next sentence: Bethanechol is for neurogenic bladder and Neostigmine is used for Ogilvie's syndrome, a sort of "neurogenic colon."

Methacholine is an **inhaled M_3 agonist**. It has no therapeutic effect, but can be used to diagnose an asthmatic who is not currently in an asthma exacerbation. There are M_1-driven secretory glands in the lung that will induce an asthma exacerbation that methacholine targets. When inhaled, methacholine induces bronchospasm, revealing the asthma.

DIRECT MUSCARINIC AGONISTS (M)	
Bethanechol	Oral = Neurogenic bladder
Methacholine	Inhaled = Bronchospasm
Pilocarpine	Drops = Eye dilation

INDIRECT DIRECT MUSCARINIC AGONISTS = AChE-i (N AND M)			
Physo Neo Pyrido	"-Stigmine"	Compete with **inhibitors** as in **myasthenia** or **organophosphate** poisoning	**Reversible**
Edrophonium		Dx myasthenia, too short acting	
Organophosphates		Lipid-soluble AChE-i	**Irreversible**

Table 9.2: Muscarinic Agonists
A list of high-yield medications, their uses, and some key notes to memorize about them.

Cholinergic Drugs #2: Indirect-Acting Muscarinic Agonists Are Acetylcholinesterase Inhibitors

Indirect-acting muscarinic agonists behave like AChR activators, but do so without binding to the AChR. That is, they increase the activity of ACh in the neural junction but **without interacting** with the receptor itself. The only way this can happen is to increase ACh concentrations in the neural junction by **blocking ACh metabolism**. This is done with **acetylcholinesterase inhibitors** (AChE-i). These are nonselective, impacting both nicotinic AChRs and muscarinic AChRs. This means that, in excess, these medications can cause massive activation of everything parasympathetic—**SLUDGE:** salivation, lacrimation, urination, defecation, GI distress, and emesis—because they stimulate both **muscarinic and nicotinic** receptors.

AChE inhibitors come in **reversible forms** (the medications we use to treat ACh-related disorders) and **irreversible forms** (organophosphates used in terrorism or pesticides). AChE inhibitors may be **lipid soluble** (capable of affecting the CNS) or may be **water soluble** (capable of affecting only neurogenic diseases of the viscera).

Organophosphates are **irreversible** and **lipid soluble**. They are not medications. They are a poison, a toxin. Because organophosphates are **irreversible**, once they bond to AChE, that enzyme is gone, permanently. This irreversible inhibition of acetylcholinesterase is called **aging**. The only way for the body to get more is to synthesize more, which takes too long compared to the toxic effects of the AChR activation. Organophosphates are used as pesticides. The insects we kill with organophosphates have a metabolism that's slow to metabolize the active, toxic form, so these chemicals kill bugs really well. Human metabolism favors the inactivation of the active, toxic form, but that toxic form can kill faster than the body can metabolize. Which means that if we can get the exposed person through the initial (and almost always fatal) phase of exposure, they might live.

Exposure is rare, coming either by way of **war and terrorism** (nerve gas) or through subacute chronic exposure to pesticides (**farmer**, **crop duster**, child eating poison from the grass).

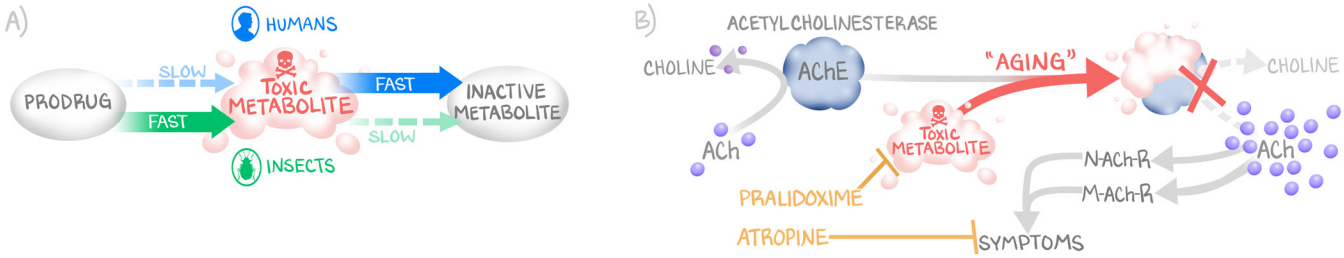

Figure 9.4: Organophosphate Biotransformation
(a) The pesticide is delivered as a prodrug. Both humans and insects metabolize that prodrug into the toxic, active metabolite. Insects favor the biotransformation into the active metabolite and are slow to metabolize to the inactive metabolite. Humans slowly biotransform the prodrug to the active metabolite, and quickly metabolize it to the inactive metabolite. (b) Therefore, by temporizing the AChR agonism with atropine, we can temporize the symptoms, keeping them alive. But to ensure that no further damage occurs, we use pralidoxime to prevent aging of AChE.

When exposure occurs, the goal is to **block the irreversible binding before it happens** using **pralidoxime**. Pralidoxime only works on AChE that has not been aged. All this does is protect the AChE from aging, allowing the natural metabolism of the organophosphate to the nontoxic metabolite without destroying the AChE. In addition, **atropine** is given as a temporizing measure, blocking the muscarinic effects that lead to symptoms, blocking the AChRs which are flooded with excess ACh. Atropine is also lipid soluble, so can follow the organophosphate, protecting nicotinic and metabotropic receptors of the CNS and periphery. Nerve gas has fallen out of favor on the exam, though the subacute exposure of the unsuspecting farmer or a child who eats poison from the yard is more likely on the exam.

There are also **reversible** AChE-is that can be used clinically. Their generic names often end in "-stimgine." **Physostigmine** is lipophilic and can cross the blood-brain barrier. This also means it has a side-effect profile closest to organophosphates. **Pyridostigmine** is used to treat myasthenia gravis and is hydrophilic. **Neostigmine** is used to treat Ogilvie's syndrome.

One AChE-i that breaks the stigmine nomenclature is **edrophonium**, which is **lipophilic** and so crosses the blood-brain barrier. But it is also very short-lived, limiting its use to the **diagnostic test** called the **Tensilon test**. If a patient with myasthenia gravis improves with edrophonium administration, then they need more AChE-i (increase the dose). If they worsen with edrophonium, their weakness may be from another disease such as infection, or they may be declining into a myasthenia crisis. It is rarely used in real practice, but is on the exam all the time.

Cholinergic Drugs #3: Muscarinic Antagonists: Acetylcholine-Receptor Blockers

These drugs bind to and compete for the acetylcholine receptor. These are labeled as muscarinic antagonists, and so are hydrophilic (except atropine), and affect only peripheral acetylcholine receptors. These drugs block the receptor, and because there is always some PNS tone and some SNS tone, these drugs will appear to boost SNS tone by reducing PNS tone—adding a muscarinic antagonist causes the opposite of receptor stimulation. There isn't an endogenous acetylcholine receptor blocker—but we can administer drugs that are. Most of these drugs will have a form of "atropine" in them, which we underline in their name.

In excess, muscarinic antagonists block all secretions. Sweat is a major source of cooling the body. Overdosing on muscarinic AChR antagonists will cause you to **dry up, heat up, and rev up**. Or, become "*blind as a bat, hot as hell, mad as a hatter, red as a beet.*" Dry mouth, blurred vision, flushing, urinary retention, and tachycardia are all side effects of muscarinic antagonism.

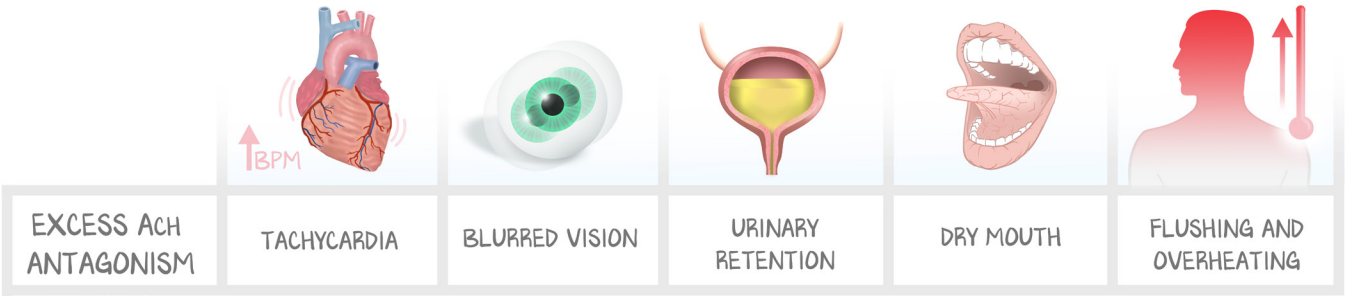

Figure 9.5: Excess Acetylcholine Antagonism
Graphical representation of the advanced organizer based on the image from Lesson #8.

Acutely, **atropine** can be used to **increase heart rate** in bradycardia and is the go-to drug for bradyarrhythmias where the AV node is still intact. This takes advantage of the M_2 receptor, but it's littered with side effects because it crosses the blood-brain barrier. We saw its use in prevention of M_3 symptoms of organophosphate poisoning as well. Because activation of M_2 slows heart rate and **slows AV conduction**, it should be avoided in bradycardias caused by high-degree blocks, as it may cause complete AV dissociation.

Ipratropium and its longer-acting derivative **tiotropium** can be used to treat **reactive airway disease** (COPD or asthma). Both of these inhaled medications cause bronchodilation by reducing smooth muscle tone of the bronchioles (or, more strictly speaking, block bronchoconstriction) by **blocking M_3 receptors**, and also have the benefit of drying up secretions by blocking M_1 receptors. Ipratropium is a

short-acting muscarinic antagonist used in COPD exacerbations; tiotropium is a long-acting muscarinic antagonist used for chronic maintenance therapy of COPD.

Scopolamine breaks the naming mold, so watch for it. It's an antiemetic, but in excess can lead to memory loss. It comes in a **transdermal patch** for motion sickness.

MUSCARINIC AGONISTS	BLIND AS A BAT HOT AS HELL MAD AS A HATTER RED AS A BEET	NICOTINIC ANTAGONISTS *NEVER IN LIFE*	
Atropine	Early treatment of organophosphates, Bradycardia, . . .	Hexamethonium	Blocks BOTH symp + para so everything goes to "default"
Ipratropium	Inhaled, Bronchodilation	Methylamine	
Tiotropium	Inhaled, Bronchodilation, Chronic COPD		
Scopolamine	Nausea, Transdermal, Memory loss		

Table 9.3: Acetylcholine Receptor Antagonists
Muscarinic antagonists and their uses and side effects. Nicotinic antagonists which will never be used except on a test question to blunt autonomic reflexes and to render indirect agonists useless. Nicotinic antagonists also block adrenergic activity, so will be used only in the situation of a test question.

Nicotinic Antagonists Are Ganglionic Blockers

You would **never** give this type of medication in real life. This class should not be considered a therapeutic medication, but rather only a drug, a laboratory chemical. **Hexamethonium** and **mecamylamine** eliminate the ganglionic synapses. **ALL EFFECTOR RECEPTORS** are still intact—cholinergic receptors and adrenergic receptors alike—which means that if a second medication is added to the system, **only direct-acting medications** will have any effect. This also means that any indirect-acting medication will do nothing—there must be the presynaptic release of neurotransmitter for indirect-acting to work.

Both SNS and PNS presynaptic ganglion neurons release acetylcholine, and both SNS and PNS postganglionic neurons receive that acetylcholine signal via nicotinic ACh receptors. That means both the SNS and the PNS are silenced, so **there will be no reflex**, only the effect of the drug.

IF YOU ADD THIS	YOU GET THIS RESPONSE
Direct agonist	Activate effector receptors
Direct agonist + antagonist	Activate effector receptors but with reduced effect
Direct agonist + another direct agonist	Activate effector receptors of both agonists

ADD THIS	GET THIS RESPONSE
Indirect agonist	No effect
Indirect agonist + antagonist	No effect
Direct agonist + indirect agonist	Only the direct agonist effect

Table 9.4:
In the presence of ganglionic blockers, the exam can add a second drug (only direct agonists will do anything) and even a third drug (variable based on the third drug added), then ask you to name those drugs.

The effect of neutralizing the reflexes sets the organs to their default. When the ANS is fully functional, the innate default of the organ is overridden by the dominant tone from the ANS. Dominant tone means either the SNS or the PNS has more signal on that particular organ. Which means each organ has a default, cannot be predicted or deduced, so comes down to strict memorization. There is no way around the memorization, as the only organs which are ever liberated from autonomic control are transplanted organs—the heart being the favorite of board examinations. Unless transplanted, where the autonomic fibers must be severed to remove the organ from the donor, no organ will ever exist in medical practice without an intact autonomic nervous system…except on the exam in the presence of a ganglionic blocker. If what follows is too complicated, memorize this one sentence:

If you give a ganglionic blocker, the **innate default** will be **low BP**, **dilated pupils**, and **increased heart rate**.

The **blood vessels** in a normal human with an intact autonomic nervous system are dominated by the sympathetic nervous system. Sympathetics induce vasoconstriction, which increases blood pressure. Applying a ganglionic blocker would remove both SNS and PNS signal. Since the dominant tone is sympathetic at rest, when both the SNS and PNS are lost, the blood vessels will appear to lose sympathetic tone. Less sympathetic tone means less vasoconstriction, and the blood pressure will fall.

The **heart** has an innate rate of about 100 bpm (when a heart is removed for a transplant, it has no nerves, and beats about 100 times a minute). In a normal human with an intact autonomic nervous system, the heart is dominated by the parasympathetic nervous system. Parasympathetics induce a decreased heart rate, which is why we say the "normal" heart rate is 60-100. If the ganglionic blocker is administered, both the SNS and PNS signals are lost, so the heart will appear to lose parasympathetic tone. Less parasympathetic tone means less decrease in heart rate, and the heart will speed up to the innate rate of ~100 bpm.

The **eyes** in a normal human with an intact autonomic nervous system are dominated by the parasympathetic nervous system. Parasympathetics induce pupillary constriction and allow for accommodation. When a ganglionic blocker is applied, both the SNS and PNS tones are lost. Since the dominant tone is parasympathetic at rest, when both the PNS and SNS are lost, the eyes appear to lose parasympathetic tone. Less parasympathetic tone results in pupillary dilation. A fixed and dilated pupil that does not constrict when light is shined on it is one test for brain death (when dead, all autonomic tone is lost).

The table below summarizes the highest-yield points of other organ systems.

SYSTEM	DOMINANT TONE	EFFECT OF GANGLIONIC BLOCKER
*Vessels	Symp	↓ SVR
Sweat	Symp	↓ Sweat
*Heart rate	Para	↑ HR
*Eyes	Para	Fixed dilated, no accommodation
GI/GU	Para	↓ Sphincter tone

Table 9.5: Effects of Ganglionic Blockers